REMARKS/ARGUMENTS

The following amendments do not affect the patentability of the subject matter or reflect changes in claim scope. The applicant reserves the right to pursue unclaimed subject matter and cancelled claims in continuing applications.

Amended Claims are Patentable

Unique methods of utilizing transcription factor decoys which encode Shear Stress Response Elements (SSRE's) Transcription Factor (TF) binding site oligonucleotides are claimed. The methods described provide a method of inducing expression of renal tubular epithilial specific genes such as megalin, cubulin, erythropoietin, and 1-α-hydroxylase. The expression of specific genes is beneficial in that renal cells, which previously only exhibited this response immediately after being isolated, can now be induced to differentiate into renal tubular epithilial cells which produce 1,25-dihydroxy-vitamin D3. We have carefully considered the recommended changes and amended the current claims accordingly. We believe the following amendments place the pending claims in condition for allowance. No new matter is added to the specification.

The claims have been appropriately re-numbered and editing clearly marked. The term "directed against nucleotides" in the specification identified SSRE sequences which bind a transcription factor (Application 09/532,001, page 5, lines 10-12). The term "directed against nucleotides" was previously removed from the pending claims. The phrase "wherein said oligonucleotide: i) is a contiguous single-stranded oligonucleotide; ii) encodes a shear stress response transcription factor binding site; and iii) encodes sequences complementary to ii." was incorporated to reflect the novel transcription factor decoy structure as described in the specification. The DNA structure is supported on page 11, lines 10-11. The addition of this phrase is lexicographic in that it merely replaces the previously amended terminology and is not intended to reflect changes in the scope of the claims.

Claims 1-3, 5, 7-10, and 27 are directed to a method of culturing cells which mimic cells found *in vivo*. Using the methods described in this application, one of ordinary skill in the art would be able to culture cells which differentiate to form structures and expression patterns, such as renal islets, which are similar to cells isolated from tissues. The use of transcription factor decoys allows the scientist to modulate shear stress response as demonstrated in the specification and distinctly claimed. Ando et al. (Jpn Heart J., Vol. 37, No. 1, Jan. 1996, p 19-32) speculate on the sequence and importance of an SSRE, but do not teach the use of SSRE sequences for modulating transcription factor activity. The embodiment of this claim is a method of using SSRE's to modulate transcription factor activity. The use of a single ODN to deliver and modulate transcription factor activity is novel. The specification has demonstrated, contrary to other teachings, that a single ODN containing an SSRE and the complement provides a method of modulating shear-stress related genes inlcuding MnSOD, ICAM, megalin, cubulin, erythropoietin, and 1-a-hydroxylase expression. The SSRE can also be used to modulate the levels of the 1,25-dihydroxy-vitamin D3 biomolecule. The specification clearly states that the SSRE regulates "genes specific for renal proximal tubular epithelial cells, including megalin, cubulin, the extracellular calcium sensing receptor, and the microvillar structural protein villin" (Application 09/532,001, page 21, line 3).

Transcription factor decoy sequences have been described in Dzau (WO199511687, published 5/4/95) and demonstrate the level of art at the time. Dzau et al. describe double-stranded ODN's which are much larger and must be delivered through liposomal vectors and/or injection, but do not describe a plausible method for large scale delivery of a transcription factor decoy. The use of single-stranded ODN's for delivery of the SSRE provides a vehicle for rapid and efficient delivery of SSRE's to the nucleus as demonstrated in the specification.

Khachigian and associates (J. Clin. Invest., Vol. 96, Aug. 1995, p 1169-1175) perform two experiments which test the binding of SSRE's by NF-κB. The first experiment included gel retardation assays with double-stranded DNA and DNAse footprinting of NF-κB complexes on SSRE elements of various sequences. Interactions

and binding could be distinguished from the present invention in that the binding occurred in nuclear extracts, not within the nucleus of a cultured cell, and the DNA was formed from separated coding and complementary sequences which could not be delivered *in vivo*. The second experiment involved the use of SSRE promoter elements to monitor reporter gene (CAT, not a shear induced protein) expression under various stress conditions. The second experiment could be distinguished from the present invention in that it uses SSRE in a plasmid (two strands of DNA) and does not involve oligonucleotide DNA. Neither involved a single-stranded ODN or nuclear delivery of a transcription factor decoy. The use of a single-stranded ODN to deliver an SSRE specific transcription factor binding site in its entirety, both coding sequence and complement, provides a novel method of altering transcription factor activity.

Applicants request consideration of the claims as amended and allowance of all pending claims.

The requisite fees, if any, are submitted herewith. Please charge fee of $420 to Deposite Acounte No. 14-0116 for a two month extension of time. Please charge additional fees or credit any overpayment to Deposit Account No. 14-0116.

Respectfully submitted,

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